REMARKS

Claims 1-7 are currently pending in the application. The foregoing separate sheets marked as "Listing of Claims" shows all the claims in the application, with an indication of the current status of each. In the specification, the paragraph beginning at page 1, line 3 has been amended.

Priority

In the specification, the paragraph beginning at page 1, line 3 has been amended to recite the number of the issued US patent corresponding to US patent application serial number 09/120,192. The present application is a divisional of US 09/120,192. The '192 application had been allowed at the time of filing of the present application, but the patent had not issued so the patent number was not known at that time. By this amendment, the number of the issued patent has been supplied.

Rejections under 35 USC § 103(a)

Claims 1-7 stand rejected under 35 USC § 103(a) as unpatentable over Kouvonen et al., (U.S. patent 5,965,458) in view of Williams et al. (U.S. patent 6,080,400) and Krivan et al. (U.S. patent 5,512,282). This rejection is traversed.

The present invention as recited in independent claims 1, 6 and 7 provides an immunoassay method, device, and kit. In these claims, what is detected is a combination of at least two substances of interest, namely: verotoxin-producing *Escherichia coli* and verotoxin; verotoxin and human hemoglobin; and verotoxin-producing *Escherichia coli* and human hemoglobin. A motivation for combining detection of these entities is given in the specification beginning on the last line of page 1 and continuing through the first three lines of page 2, which states that methods known for detecting verotoxin and the *E. coli* that produces it, at the time of filing of the application, were restricted to single tests that did not detect the two concurrently. In addition, the methods available at that time required enrichment before detection, and were thus time-consuming and required complicated manipulation. In contrast, the present invention provides a convenient, relatively simple way to detect a combination of at least two of verotoxin-producing *Escherichia coli,* verotoxin, and human hemoglobin. Attached as Exhibit 1

are the results of applying the assay kit to clinical isolates. Examples 1-16 represent the results of detection of E. Coli and/or verotoxin, and/or hemoglobin, which may be present without concurrent infection.

Kouvonen et al. (hereafter "Kouvonen") describe a test strip for rapid immunoassays. The strip is illustrated, for example, in Figure 1A and 1B. The test strip preferably has "two supporting covers or backings, one on either side" column 3, lines 49-50). One backing (**1** of Figure 1B) has located thereon two pads **3** and **5**, one of which (**3**) has a zone **7** on which dried labeled reagents are placed. A test membrane **2** is located between pads **3** and **5** and contains substances needed to carry out the reaction that is being detected by using the test strip. The precise location of test membrane **2** with respect to backing **1** and pads **3** and **5** is the key feature of the invention of Kouvonen. There is a gap **4** between pads **3** and **5**, and membrane **2** is placed over gap **4** without filling the gap. Gap **4** constitutes "...a small chamber, open on both sides, where the liquid flow on membrane **2** is not disturbed..." (column 5, lines 6-9). This is the essence of the invention of Kouvonen. The introduction of such a gap/chamber in the test strip purportedly confers advantages over similar strips with other designs with respect to allowing the reactants that are flowing up the strip to mix well in the undisturbed, sheltered chamber, which is designed to be small enough so that the reactants, after mixing, will continue flowing out of the chamber and up the strip. Applicant submits that the inventive concept of Kouvenen is thus limited to the design of a test strip with a gap/chamber that is said to allow better mixing of the reagents and test substances being assayed with the strip. Thus, although Kouvenen discusses various uses for the test strip (e.g. column 8, lines 4-67 and column 9, lines 1-30) there is absolutely no discussion of detecting any particular type of entity. The Examples include detection of an extremely heterogenous group of entities, including hCG (pregnancy test), insulin-like growth factor binding protein-1, hemoglobin, toluene, and *Salmonella* from a wide variety of sources (fecal matter, soil, and food). Clearly, the test strip of Kouvenen is intended for generic use. In particular, Applicant notes the complete lack of discussion concerning the detection of any useful <u>combination(s)</u> of substances of interest. Instead, Kouvenen describes an "all-purpose" strip with a unique design. In particular, <u>and as recognized by the Examiner</u>, Kouvenen does <u>not</u> teach or refer at all to the detection of verotoxin or verotoxin producing

Escherichia coli, and certainly does not allude to testing for these two substances together on the same test strip. Applicant submits that knowledge of the work of Kouvenen would not have provided one of skill in the art with motivation to develop any particular combination(s) of entities to assay with the test strip. In particular, there would be no reason to single out verotoxin and the bacterium that produces it, either together or in combination with any other entity (e.g. hemoglobin) given a knowledge of the work of Kouvenen.

The Examiner has cited Williams et al. (hereafter "Williams") as supplying this deficiency by disclosing a method for detecting verotoxin produced by *E. coli* using an immobilized antibodies directed against the verotoxin. The Williams invention is largely concerned with new methods of producing antibodies to verotoxin. Prior to the work of Williams, obtaining relatively large quantities of verotoxin subunits that retained biologically correct conformations, and that would thus be suitable for eliciting an immune response in a host from which antibodies could be isolated, was problematic (see column 22, lines 5-8, and lines 26-43). The invention is entirely focused on this problem and its solution. Williams provides methods for expression of large quantities of individual subunits of verotoxin in the periplasmic space of *E. coli.* The subunits can be purified and reassembled into holotoxin *in vitro* and used to immunize a host organism to produce antibodies. Particularly favored host organisms are non-mammals such as avian species. While Williams demonstrates overcoming a major hurdle to producing verotoxin antibodies, and contemplates using the antibodies in diagnostic tests to detect verotoxin, there is no discussion or showing that a detection system allowing the detection of any particular combination of the detection of verotoxin with other entities would be advantageous. In particular, Williams does not show or suggest the detection of verotoxin together with the simultaneous detection of the bacterium that produces the verotoxin. The "solid support" methodologies advocated by Williams are described only in a cursory manner ("cartridges, columns, beads, or any other solid support medium", column 31, lines 25-27). A knowledge of the work of Williams would not provide motivation for one of skill in the art to utilize the relatively complicated test strip of Kouvenen over any other solid support. In fact, Williams focuses on a stationary "sandwich assay" in which the antibody is immobilized in the wells of a microtiter plate (see Example 3 and Example 10C). In a sandwich assay, there is no

mobile phase, and thus no need for a test strip that allows migration of substances along the strip. Thus, there would have been no motivation in either of these references for those skilled in the art to combine the two references. In particular, neither reference provides the impetus to simultaneously detect verotoxin and the bacterium that produces it, either together, or in combination with another entity such as hemoglobin.

Krivan et al. (hereafter Krivan) discloses monospecific polyclonal IgG-type antibodies to shiga-like toxins (SLTs, also known as verotoxins) and methods for producing large quantities of the antibodies. In particular, Krivan "...discovered that pregnant cows immunized with purified SLTs produce monospecific, polyclonal antibodies to SLTs that are of a surprisingly and unexpectedly high titer" (column 5, lines 42- 44). The cows exhibited no ill effect from immunization with active, native toxin. Thus, the antibodies that were produced recognize epitopes that are encountered in a realistic situation, i.e. epitopes exhibited by active, native toxin molecules. Such antibodies should be more effective than those produced to denatured, inactive toxin molecules, which are usually used to generate toxin antibodies in order to minimize ill effects of the toxin on the immunized host animal. Interestingly, the antibodies are present in the colostrum and milk of the immunized cows, providing a convenient source of the antibodies, either for direct consumption, or as a source for further isolation and purification. Krivan states that one possible use of the antibodies is to "...detect the presence or concentration of an SLT, or the presence of SLT-producing bacteria, in a sample..." (Column 6, lines 52-54). However, use of the antibodies for such a purpose would in fact provide direct evidence only for the presence of the toxin. The presence of the bacteria that produced the toxin could be assumed only by inference ("toxin is present so the bacteria that produce it must also be present") because the antibodies react only with the toxin molecule itself, and not with the bacteria. In fact, there is no showing or suggestion in Krivan that detection of a combination of toxin and the bacteria that produces it would be possible or advantageous. Further, there is no suggestion that the methods of detection of Krivan should be carried out with a test strip such as that designed by Kouvonen, over any other available solid phase method, or in conjunction with the use of yet another antibody to the toxin as taught by Williams. The preferred embodiment described by Krivan is an ELISA reaction, preferably a receptor-based ELISA. An alternative method is the attachment of

the antibodies to "solid particles" and detection of agglutination (column 6, lines 58-63). In the kit described by Krivan, the preferred solid support is the well of a microtiter plate (column 6, line 67-column 7, linel and column 7, lines 12-13). There is no preference shown or discussed for the use of a test strip. Further, Applicant notes that both Williams and Krivan supply teachings regarding the production of antibodies for the detection of verotoxin alone. Neither provides reagents or methods for the detection of an *E. coli* bacterium that produces the toxin. Thus, neither reference can be said to show or suggest direct detection of both verotoxin and the bacteria that produce it in a single test. Further, neither reference supplies a teaching or motivation for one of skill in the art to utilize a test strip such as that described by Kouvenen. Applicant notes that if a combination of the three cited references was developed, the result would be the test strip of Kouvenen on which two different antibodies, both of which were specific for verotoxin and only verotoxin, would be present, i.e. the result would not be the present invention as recited in independent claims 1,6, and 7 of the present application, which require the simultaneous detection of at least two substances, for example, the combinations of verotoxin-producing *Escherichia coli* and verotoxin; verotoxin and human hemoglobin; and verotoxin-producing *Escherichia coli* and human hemoglobin. Thus, no combination of Kouvenen, Williams and Krivan could have rendered the subject matter of the present invention obvious at the time of filing of the present application.

In view of the foregoing, Applicant respectfully requests reconsideration and withdrawal of this rejection.

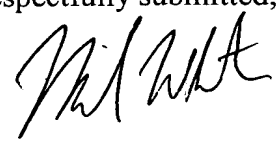
Concluding remarks

In view of the foregoing, it is requested that the application be reconsidered, that claims 1-7 be allowed, and that the application be passed to issue.

Should the Examiner find the application to be other than in condition for allowance, the Examiner is requested to contact the undersigned at 703-787-9400 (fax: 703-787-7557; email: ruth@wcc-ip.com) to discuss any other changes deemed necessary in a telephonic or personal interview. If an extension of time is required for this response to be considered as being timely filed, a conditional petition is hereby made for such extension of time. Please charge any

deficiencies in fees and credit any overpayment of fees to Attorney's Deposit Account No. 50-2041 (Whitham, Curtis & Christofferson).

Respectfully submitted,



Michael E. Whitham
Reg. No. 32,635

Whitham, Curtis & Christofferson, P.C.
11491 Sunset Hills Road, Suite 340
Reston, VA 20190
703-787-9400
703-787-7557 (fax)

◎ Clinical test

analyte	Evaluation results															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
O157	+	+	+	+	-	+	+	-	+	-	-	+	-	-	-	-
VT1	+	+	+	-	+	+	-	+	-	-	+	-	+	-	-	-
VT2	+	+	-	+	+	-	-	-	+	+	+	-	-	+	-	-
Hb	+	-	+	+	+	-	+	+	-	+	-	-	-	-	+	-

1. onset due to VT 1- and VT 2-producing O157
possibility to become serious due to VT 2
3. onset due to VT 1 alone-producing O157
4. onset due to VT 2 alone-producing O157
possibility to become serious due to VT 2

2. infected with VT 1- and VT 2-producing O157 (no onset)
6. infected with VT 1 alone-producing O157 (no onset)
9. infected with VT 2 alone-producing O157 (no onset)
12. infected with VT non-producing O157 or with virus having cross reactivity (no onset)

5. onset due to VT 1- and VT 2-producing EHEC (enterohaemorrhage *Escerichia coli*) other than O157, possibility to become serious due to VT 2
8. onset due to VT 1-producing EHEC other than O157, or Shiga bacillus
10. onset due to VT 2-producing EHEC other than O157,
possibility to become serious due to VT 2

11. infected with VT 1 and VT 2-producing EHEC other than O157 (no onset)
13. infected with VT 1-producing EHEC other than O157, or Shiga bacillus (no onset)
14. infected with VT 2-producing EHEC other than O157 (no onset)

7. hemorrhage due to disorder of digestive tract, though suspected of infection with O157

15. disorder of digestive tract

16. healthy

◎ food test

Analyte	Evaluation results							
	1	2	3	4	5	6	7	8
O157	+	+	+	-	+	-	-	-
VT1	+	+	-	+	-	+	-	-
VT2	+	-	+	+	-	-	+	-

1. presence of VT 1 and VT 2-producing O157
2. presence of VT 1 alone-producing O157
3. presence of VT 2 alone-producing O157

4. presence of VT 1 and VT 2-producing (O-26, O-111)
5. presence of toxin non-producing O157 or bacterial cell having cross reactivity
6. presence of VT 1 alone-producing EHEC, or Shiga bacillus
7. presence of VT 2 alone-producing EHEC

8. EHEC negative